B111 The Development of an X-Chromosome Insertion-Deletion (InDel) Multiplex for Forensic Applications

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After attending this presentation, attendees will have a better understanding of the development and application of the X-chromosome InDel marker system for use with forensic DNA samples.

This presentation will impact the forensic science community by providing results from a developed novel X-chromosome InDel as a key aspect for the analysis of special forensic investigations as it can contribute to traditional DNA testing systems in cases in which those systems do not produce results due to the presence of degraded DNA.

Short Tandem Repeats (STR) loci are commonly used genetic markers for the purpose of forensic identification; however, there are many situations in which a full DNA profile cannot be achieved from a crime scene due to degradation of the biological samples over time. Recently, there is a tendency toward Single Nucleotide Polymorphism (SNP) and InDel among forensic scientists since these markers have smaller DNA sequences; therefore, a successful DNA profile can be achieved from even a small amount or degraded biological samples. InDel polymorphism is a type of genetic variation that is formed by the addition or loss of one or several bases in the human genome. InDel loci have small (60bp to 200bp) amplicon length (Polymerase Chain Reaction (PCR) products) and have begun to be used in forensic identification. X-chromosome InDel polymorphism can be used in specific kinship investigations (incest cases, etc.).[1-5] The goal of this study is to develop a multiplex InDel panel, consisting of 18 X-chromosome loci with a short amplicon size, for forensic purposes.

Eighteen X-chromosome InDel markers were chosen based on the following criteria: (1) mean heterozygosity of more than 30% in Europeans; (2) a minimum of 250Kb differences between each InDel loci; and, (3) the amplicon length was less than 300bp. InDel primers were designed using the PRIMER3 software and tested for hairpin and primer-dimer secondary structures with the AutoDimer software. Singleplex PCR was applied to the InDel loci, then multiplex PCR was performed containing PCR master mix, optimized primer concentrations, and 1ng-10ng of genomic DNA. InDel loci fragments were separated using an ABI® PRISM® 310 Genetic Analyzer and analyzed with GeneMapper® v3 software. The validation study of these 18 InDel loci was performed with the following parameters and aspects: analytical threshold, sensitivity and stochastic threshold, heterozygous balance, precision and accuracy, repeatability and reproducibility, genotype concordance (9947a), DNA mixtures, and case samples.

Eighteen InDel loci were successfully amplified using a single multiplex reaction. Optimization and validation parameters of the 18 InDel Multiplex was successfully applied. The high performance of this 18 InDel multiplex fluorescent PCR system makes it a valuable additional system to the current STR systems and can be used for forensic purposes.

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X-Chromosome, InDel, Panel Development